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CHANGES IN THE BACTERIOLOGY OF ULCERATIVE COLITIS WHEN BLOOD IS PRESENT

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In the literature up to the beginning of this century the etiology of ulcerative colitis was invariably mentioned as "cause unknown" and the disease described as "idiopathic" or "nonspecific ulcerative colitis," etc. Beginning about 1900, the literature showed evidence of a growing belief in a bacterial cause for ulcerative colitis, although articles occasionally reported a negative infectious cause. Throughout the literature on ulcerative colitis there are many different theories of etiology and conflicting bacteriologic studies. This finds expression in the words of Sir Samuel Wilks (1875), who first described the disease: "There is * * * difficulty in distinguishing dysentery from simple colitis by anatomical characters. Dysentery, in fact, produces a variety of colitis distinguished from others by a special course, and by clinical phenomena; though when the dysenteric fever has passed off, the state of the colon which is left is much the same as that of simple ulceration."

While many believe that ulcerative colitis is an infectious condition, there is little unanimity as to the nature of the infection.

Leusden (1921), Hurst (1931), and Einhorn (1923), have suggested that the disease was due to some form of dysentery bacillus. Rolleston (1923) thought that the normal intestinal bacteria could, under favorable conditions, become pathogenic. Rankin, Bargen and Buie (1933) and others, from cultures made from the bases of ulcers, animal experimentation and the examination of tissue, attach etiological significance to a gram positive diplococcus cultivated by a special method. This they reported as having been recovered from approximately eighty per cent of the cases at the Mayo Clinic. A large number have added confirmation to Bargen's work mainly as to the significance of this organism among whom are Soper (1927), Portis (1927) and Garrod (1931). Mackie (1932) from the seventy-five per cent incidence of the Flexner-Y bacillus in the transitory diarrheas during the late war drew attention to this organism.

There is no doubt that in this country cases are met with in which it is most logical that the Flexner-Y or Shiga or some types of Bacillus dysenteriae are important as the initial cause of ulcerative colitis. In most instances there has been a history of personal contact with various parts of the world where bacillary dysentery was endemic. Bastedo (1924) suggests the possibility of true bacillus dysentery infections in the ordinary cases met with in the United States. It is possible that this type of infection has a major role in the cause of ulcerative colitis in this country where large quantities of foods, such as dried fruits, etc., are imported from countries in which such infections are endemic. However, the possibility of bacillary dysentery seems remote when these dried foods are well cooked before being eaten. Since the cultural methods of examination of the true dysentery organisms are so well known and employed, it is of interest to note that with the large number of cases of ulcerative colitis met with in this country these organisms are too infrequently encountered and reported.

It occurred to the writer that there must be some reason for this mass of conflicting theories on the etiology of such a prevalent disease as ulcerative colitis. I noted that when feces from ulcerative colitis patients had demonstrable blood present there was always a marked increase in gram positive organisms, the approximately normal ratio of gram positive and gram negative organisms being three to seven. The ratio of gram positive and gram negative organisms can be controlled, under normal conditions, by the diet. A high protein diet will increase the gram negative organisms while a high carbohydrate diet will stimulate the opposite group.

The purpose of the experiments was to establish a condition as

near as possible to that which exists in the normal intestinal tract and then introduce defibrinated blood. After noting the changes in the normal ratio of gram positive and gram negative organisms, the writer decided to investigate what influence defibrinated blood would have on certain normal and pathological organisms. These experiments worked so well in test tubes that he decided to extend the problem to investigate the results of inoculating animals with pathological organisms and observe the changes that took place in the intestinal tract.

Apparently living bacteria have two buffering mechanisms. Shanghnessy and Winslow (1927) found that, in solutions more alkaline than the reaction of optimun viability, B. coli liberates acid substances, while in solutions more acid than this reaction, the organism liberates alkaline substances.

There have been a number of reports of a final limiting pH produced by growing cultures, which was originally thought to be characteristic of the culture and regarded as a "physiological constant" of the organism (Michaelis and Marcora, 1912; Clark, 1915; Ayers, 1916; et al.). It was later realized that the factors controlling this final pH are numerous. It depends, according to Shunk and Wolf (1921), not only on the kind of organism, but also on (a) initial reaction of medium, (b) kind and concentration of acid or buffer used in adjusting the reaction of the medium, (c) kind and concentration of fermentable carbohydrate, (d) food accessories, and (e) physical state of the medium. The writer made no attempt to control the final pH of the medium but in each instance the initial cultures were adjusted to approximate physiological normals.

All of the defibrinated blood used in the experiments was obtained from an individual who had a normal ratio of gram positive and negative organisms in his feces. This defibrinated blood contained .576 per cent chlorides estimated as sodium chlorides. Winslow, Walker and Sutermeister (1932) stated that 0.1 molar sodium chloride acts as a mildly stimulating medium and has less toxic effect on the growth of bacteria. A 0.1 molar sodium chloride is equal to a .5845 per cent solution. The defibrinated blood contained an average of .112 per cent sugar (dextrose). The average age of the blood when used was six hours.

The defibrinated blood used in the cultures was diluted with nutrient broth which contained .58 per cent sodium chloride and

.11 per cent dextrose and was adjusted to a pH of 7.2 for the B. coli communis cultures, pH 7.8 for the B. dysenteriae Flexner cultures and pH 7.4 for the B. lactis aërogenes cultures.

The literature contains a number of different methods for determining the approximate number of bacteria present in a given sample. McCrady (1915) introduced a method for determining the number of bacteria per cubic centimeter by culturing small samples of the original specimen. McCrady later (1918) developed the equations from which it is possible to calculate the most probable number of organisms per cubic centimeter from data obtained by inoculating a series of tubes with the same dilution or from several series of tubes inoculated with several different dilutions. To simplify the use of the method, McCrady (1918) has solved the equations for all possible combinations for a number of special cases. These results have been put into tables so that the method may be used without tedious calculation.

However, to minimize the possible error in calculating the number of bacteria per cubic centimeter present in the defibrinated blood cultures, the writer utilized the formula developed by Halvorson and Ziegler (1933). Halvorson and Ziegler approached the problem from a different angle and worked out tables somewhat analogous to those of McCrady. They found that when three effective dilutions are used to determine the bacterial content of cultures, the accuracy is independent of the number of organisms, and dependent only on the number of tubes used in each dilution. They showed that there is a marked decrease in errors as the number of tubes of each dilution is increased, reaching a point where symmetrical distribution is produced. This point was demonstrated to be slightly greater than forty tubes.

For the determination of the number of B. coli communis present at the different time intervals, forty fermentation tubes of nutrient broth which contained 1.0 per cent lactose were inoculated with 0.01 c.c. of the suspension, forty tubes with 0.001 c.c., and forty tubes of 0.0001 c.c.

For the determination of the number of B. dysenteriae Flexner present at the different time intervals, forty fermentation tubes of nutrient broth which contained 1.0 per cent maltose were inoculated with 0.01 c.c. of the suspension, forty tubes with 0.001 c.c., and forty tubes of 0.0001 c.c.

CHART No. 1

B. Coli communis

Medium	Initial Culture	12 Hrs.	24 Hrs.	36 Hrs.	48 Hrs.
5 ml. Defibrinated blood 1 ml. B. Coli communis suspended in nutrient broth	5,000	3,800	3,000	2,300	1,750
5 ml. of a 1/5 dilution of defibrinated blood 1 ml. B. Coli communis suspended in nutrient broth	5,000	3,200	1,400	800	0
5 ml. of a 1/10 dilution of defibrinated blood 1 ml. B. Coli communis suspended in nutrient broth	5,000	1,850	0	0	0
5 ml. of a 1/10 dilution of defibrinated blood		+BCc	+BCc	—BCc	—BCc
.5 ml. B. Coli communis	2,500				
.5 ml. of normal feces		+GpC	++GpC	+++GpC	++++GpC
5 ml. of nutrient broth		8+BCc	7+BCc	7+BCc	7+BCc
.5 ml. B. Coli communis	2,500				
.5 ml. of normal feces		2+GpC	3+GpC	3+GpC	3+GpC

0=no evidence of organism present
+=relative value.
—=negative for specific organism.
BCc=B. Coli cummunis.
GpC=Gram positive cocci.

For the determination of the number of B. lactis aërogenes present at the different time intervals, forty fermentation tubes of nutrient broth which contained 1.0 per cent saccharose were inoculated with 0.01 c.c. of the suspension, forty tubes with 0.001 c.c., and forty tubes of 0.0001 c.c.

CHART No. 2

B. dystenteriae Flexner

Medium	Initial Culture	12 Hrs.	24 Hrs.	36 Hrs.	48 Hrs.
5 ml. Defibrinated blood 1 ml. B. dysenteriae Flexner suspended in nutrient broth	5,000	4,000	3,800	3,100	1,800
5 ml. of a 1/5 dilution of defibrinated blood 1 ml. B. dysenteriae Flexner suspended in nutrient broth	5,000	4,500	3,600	2,000	900
5 ml. of a 1/10 dilution of defibrinated blood 1 ml. B. dysenteriae Flexner suspended in nutrient broth	5,000	3,800	2,100	600	0
5 ml. of a 1/10 dilution of defibrinated blood .5 ml. B. dysenteriae Flexner .5 ml. of normal feces	2,500	+BdF +GpC	+BdF ++GpC	+BdF +++GpC	−BdF ++++GpC
5 ml. of nutrient broth .5 ml. B. dysenteriae Flexner .5 ml. of normal feces	2,500	+BdF +GpC	++BdF ++GpC	+++BdF ++GpC	+++BdF +++GpC

0=no evidence of organism present
+=relative value.
−=negative for specific organism.

BdF=B. dysenteriae Flexner.
GpC=Gram positive cocci.

Each of four rabbits was given, with the aid of a catheter direct to the stomach, 5,000 B. dysenteriae Flexner organisms suspended in nutrient broth. After an elapse of twenty-four hours one of the rabbits was killed and autopsied. The contents of the small and large gut were negative for blood. The ratio of gram negative to gram positive organisms in the small gut was nine to one and in the large gut the ratio was eight to two. B. dysenteriae Flexner was demonstrated in both the small and large gut. In the small gut there were a large number of small ulcerated areas. Tissue sections of these ulcerated areas showed numbers of gram negative organisms but no gram positive organisms.

CHART No. 3

B. Lactis aërogenes

Medium	Initial Culture	12 Hrs.	24 Hrs.	36 Hrs.	48 Hrs.
.5 ml. Defibrinated blood 1 ml. B. Lactis aërogenes suspended in nutrient broth	5,000	3,700	3,100	2,100	1,400
5 ml. of a 1/5 dilution of defibrinated blood 1 ml. B. Lactis aërogenes suspended in nutrient broth	5,000	3,400	2,700	1,200	70
5 ml. of a 1/10 dilution of defibrinated blood 1 ml. B. Lactis aërogenes suspended in nutrient broth	5,000	2,000	50	0	0
5 ml. of a 1/10 dilution of defibrinated blood		+BLa	—BLa	—BLa	—BLa
.5 ml. B. Lactis aërogenes	2,500				
.5 ml. of normal feces		+GpC	++GpC	+++GpC	++++GpC
.5 ml. of nutrient broth		9+BLa	8+BLa	7+BLa	7+BLa
.5 ml. B. Lactis aërogenes	2,500				
.5 ml. of normal feces		2+GpC	2+GpC	2+GpC	3+GpC

0=no evidence of organism present
+=relative value.
—=negative for specific organism.
BLa=B. Lactis aërogenes.
GpC=Gram positive cocci.

After an elapse of thirty-six hours another rabbit was killed and autopsied. The contents of the small and large gut were positive for blood. The ratio of gram negative to gram positive organisms in the small gut was six to four and in the large gut was seven to three. B. dysenteriae Flexner was demonstrated in both the small and large gut. Throughout the intestinal tract there were large numbers of ulcerated areas. Tissue sections of these ulcerated areas showed both gram negative and gram positive organisms.

After an elapse of forty-eight hours the remaining two rabbits were found dead. There were a number of perforated areas in the

intestinal tract. The peritoneal cavities were distended with a light brown pus. This pus after extensive culturing showed no B. dysenteriae Flexner, but on all occasions gram positive organisms were found. The contents of the intestinal tract of both animals were positive for blood. The average ratio of gram negative to gram positive organisms in the small gut was two to eight and in the large gut three to seven. Sections from well developed ulcers showed gram negative and gram positive organisms. Sections from small ulcerated areas of the large gut showed only gram negative organisms. Sections from the perforated areas showed gram positive organisms but no gram negative organisms.

During the course of these experiments there has always been present the symbiosis factor which appears to be upset when blood is present. Far too little work has been done and too little attention paid to the symbiosis factor in infections. It is well known that certain organisms multiply freely in the presence of others, but there seem to be some enhancement properties that affect the existence of one in the presence of the other. The large number of organisms present in the normal and pathological intestinal flora opens a limitless field for investigating symbiotic problems.

Conclusions

1. Defibrinated blood inhibits the growth of gram negative organisms.
2. The normal symbiosis of organisms is altered by the presence of extra-vacular blood.

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THE A. S. C. L. T. QUESTIONNAIRE*

1. GENERAL INFORMATION

By SISTER M. JOAN OF ARC WILSON, R.S.M., M.T., AND CHARLES E. BRAMBEL, Ph.D.

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In an endeavor to contact as many technicians as possible, and at the same time to ascertain the nature of the work in which they are engaged, and the various methods used, a questionnaire was sent to our Society members, and also to the hospitals accredited by the American College of Surgeons. It was hoped that through this medium a better spirit of co-operation and understanding would be fostered among the technicians, and that the information so collected would be valuable to those whose library facilities are rather limited. Furthermore, considerable time would be saved on the part of technicians in small, isolated laboratories, by showing them the general trend of present methods in a representative cross section of institutions.

The questionnaire had its origin from a suggestion by Miss Sarah McCarty (President 1934-35), and from this proposition developed the definitive questionnaire.

The first page of the questionnaire asked for general information relative to the laboratory staff and also specific inquiries concerning the June 1935 Convention, such as the possible attendance, and solicitation for scientific papers and exhibits. A summary of the data collected is presented in this paper.

The second page covered the chemical, macro- and microscopic methods for body fluids and excreta, as well as special chemical estimations for the nitrogenous compounds in such material. Page three dealt with hematology, including routine and special determinations; and page four, with the preparation and procedures for blood transfusions. The fifth sheet was devoted to bacteriology, asking for culture methods for routine inoculations, and for the organisms requiring selective media. On page six were questions covering the methods for twenty-two bio-chemical determinations, including anticoagulants and preservatives. The questions on the

*This is the first of a series of articles to appear in the Bulletin outlining the information obtained from the questionnaire and giving graphs pertaining to same.

seventh sheet were segregated into the following groups: (1) serological procedures; (2) methods for histological technic; (3) general animal inoculations; and (4) miscellaneous.

When we discovered the area we hoped to cover contained two thousand three hundred and ninety-two destinations, we realized that it would be necessary to have many co-operators on this committee. Hence the following members were appointed to address and mail the papers:*

Miss Elizabeth Cramer, 164 Market Street, Lexington, Ky.

Miss Helen Hanley, Parke, Davis & Co., Detroit, Mich.

Miss Frieda Ward, St. Barnabas Hospital, Newark, N. J.

Miss Dorothea Zoll, Lankenau Hospital, Philadelphia, Pa.

Sr. Mary Claude, Mercy Hospital, Baltimore, Md., who also took care of mimeographing.

The following were appointed to summarize the returns:

		Responses Received
Page 1.	Sister Mary Joan, Mercy Hospital, Baltimore, Md.	424
Page 2.	Miss Luella Gifford, 339 Boush St., Norfolk, Va.	400
	Miss Faith Dravis, Grandview Hospital, La Crosse, Wis.	400
Page 3.	Miss Mabel Varner, Methodist Hospital, St. Louis, Mo.	380
Page 4.	Miss Myrtle Sand, 1850 W. Jackson Blvd., Chicago, Ill.	410
Page 5.	Miss M. Eleanor Behr, Mercy Hospital, Baltimore, Md.	388
Page 6.	Miss Anna Wassell, St. Joseph's Infirmary, Savannah, Ga.	388
Page 7.	Miss Irene Satterfield, 803 Medical Arts Bldg., Birmingham, Ala.	395

Within a short time after the papers had been mailed, further stimulus was given through the hundreds of letters received by the officers and the committee, expressing intense interest and containing numerous inquiries about our organization and its activities.

Considering the length of the questionnaire, the infancy of the Society sending it, and the fact that not only was no return postage

enclosed, but rather the answers required seven different destinations, we were more than gratified with the twenty per cent return, or an average of over four hundred responses, from institutions ranging from six to ten thousand beds, besides the various laboratory groups.

Our mailing list included fifty-five territories, as shown in Figure 1, which also gives the returns according to location. The heavy black blocks indicate the number of questionnaires sent to each territory, and the white blocks the number responding.

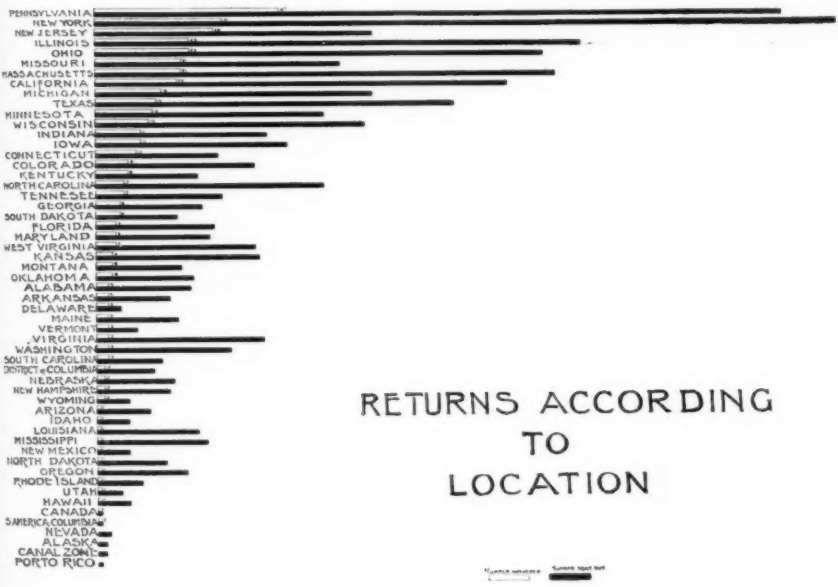

Figure 1

While Pennsylvania heads the list with fifty-five returns, or twenty-six per cent, some states reached a higher figure; namely, New Jersey with forty-two per cent, and Delaware with fifty per cent. From Canada and South America one hundred per cent were returned, and one response came from Hawaii.

Figure 2 shows the general returns from each page of the questionnaire according to the bed capacity.

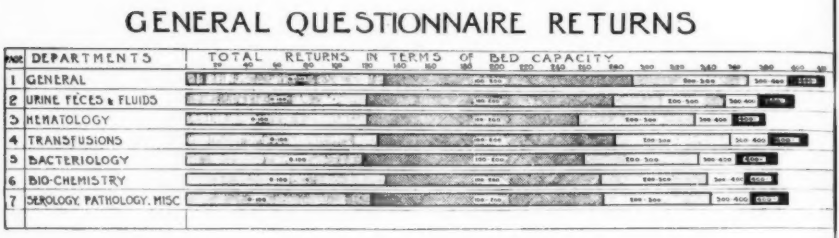

Figure 2

The following table gives the bed capacity, number of technicians and volume of work reported. Due to the lack of standardization in counting tests unavoidable discrepancies are probably present.

Bed Capacity	Number Institutions	Number Technicians	Number Tests Per Year	Average Tests Per Year
0-100	132	1- 6	865- 42,000	6,226
100-200	155	1- 6	1,000- 31,000	13,955
200-300	77	1-17	6,600- 55,000	23,343
300-400	27	1- 7	7,718-100,000	24,613
400-500	13	1-10	4,000-100,000	51,152
500-10,000	15	1-22	4,000-281,000	58,416

This table indicates a great variation in the number of technicians employed by different institutions of the same bed capacity, and a still wider range in the actual number of tests reported from the same groups.

Of the seven hundred and eight technicians whose names were sent in, two hundred and eighty-two or thirty-nine and eight-tenths per cent were registered. The work in which they are engaged was reported as follows:

General laboratory work	54%		
Not stated	21%		
Specialized	25%	- Bacteriology	4%
		Histology	3
		Chemistry	3
		Hematology	2.4
		Serology	.8
		Urinalysis	.7
		Public Health	.4
		Misc.	1.4
		Two or more subjects	9.3

The interne's association with the laboratory was reported as follows:

None in laboratory	47.7
None in institution	7.8
Night work and emergency	23.8
Ward routine and emergency	9.7
General work	5.7
Occasional misc. tests	5.1

In seventy-nine per cent of the institutions the technician is responsible for Sunday and holiday work; in nineteen per cent the internes are responsible, and two per cent reported being closed on these days.

About one-half of the institutions reported the use of Todd and Sanford's "Clinical Diagnosis by Laboratory Methods," and also Kolmer and Boerner's "Approved Laboratory Technic" as reference books. In addition to these two standard texts there were two hundred and six other reference books and four journals mentioned.

By solicitation on the questionnaire an opportunity was given the technicians to state just what they were interested in having discussed at the June Convention, and two hundred and twenty-six availed themselves of this suggestion; thirty-two were interested in diverse phases of chemistry; fifty-seven in hematology; thirty-six in bact logy; eighteen in excreta and fluids; eight in tissue work; six in serology, and sixty-nine in miscellaneous topics. Eighty-five different subjects were mentioned in this list. This was a valuable index in formulating our program and we tried to cover as much of the desired information as possible.

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*The authors take this opportunity to again express their sincere appreciation and grateful thanks for all the splendid co-operation received from each member of the committee who so generously helped, at a great sacrifice of time, to bring this work to a successful issue.

Very special thanks are due to Sister M. Judith, O.S.B., to whom we are indebted for the excellent drawings of the graphs.

COLLOIDAL GOLD

By JOHN J. PERKINS, L.T.

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In 1901 Zsigmondy[1] developed the principle that solutions of electrolytes or of proteins precipitate colloidal gold, but when both electrolytes and proteins are present the gold is not precipitated. Lange[2] in 1912 applied this principle to spinal fluids in an attempt to determine the nature of the proteins in the spinal fluid. As the result of his observations we have the well-known colloidal gold test which has proved of inestimable value and is a matter of routine in practically every clinical laboratory. A brief review of the medical literature will reveal numerous methods for the preparation of colloidal gold. Each method claims its advantages over the other, while they all have one decided disadvantage, and that is the failure to produce consistently and uniformly solutions which will react properly with known paretic and normal spinal fluids, without previous adjusting to what is termed neutrality. In other words, all present methods for the preparation of this reagent tend to produce solutions that are too alkaline or too acid in reaction, thereby decreasing or increasing its sensitivity when tested with the spinal fluid. The apparent cause for this discrepancy in the reaction of colloidal gold solutions is due to the failure to control the electrolytic forces involved in the preparation of the gold solution.

The technic of Miller, Brush, Hammers and Felton[3] for the preparation of colloidal gold is widely used and is fairly constant in its results. However, the finished reagent is very often found to be useless because it will not react properly with paretic fluids. The inconsistency of this method is probably due in a large measure to the formation of condensation products of dilute formaldehyde in dilute alkaline solution. These products are a mixture of sugar-like substances which are termed formoses. Under certain conditions the formoses give a resinous substance which acts as a good protecting colloid for the gold. Weston,[4] while working in this laboratory used all the methods for the preparation of colloidal gold and like others found that at times each method failed. Everything concerned in making this reagent has been accused of causing failure to make satisfactory solutions. The following technic for the preparation of colloidal gold is substantially a further modification of Haden's[5] modification of the original Mellanby and Anwyl-Davies[6] technic. This technic has been in use in this laboratory for over one year and has proved itself absolutely dependable.

TECHNIC FOR PREPARATION OF COLLOIDAL GOLD

REAGENTS:

1. 1 per cent solution of gold chloride, $AuCl_3HCl.3H_2O$, acid yellow, (Merck's Blue Label), highest purity.
2. 1 per cent solution of potassium oxalate, $K_2C_2O_4.H_2O$, highest purity. (Weigh on analytical balance.)
3. 1 per cent solution of potassium hydroxide, highest purity. Most conveniently prepared by dilution of a more concentrated solution.
4. "Buffer" mixture, prepared as in "a" or "b".
   (a) Weigh accurately on analytical balance 7.262 gm. of disodium hydrogen phosphate, *$Na_2HPO_4+2H_2O$, and to this add 2.376 gm. of Sorensen's potassium phosphate, KH_2PO_4. Mix thoroughly in mortar with pestle. This mixture will give a Ph of 7.4.
   (b) Weigh on balance 5.792 gm. of Sorensen's dibasic—anhydrous sodium phosphate, and to this add 2.376 gm. of Sorensen's monobasic—anhydrous potassium phosphate. Mix thoroughly in mortar with pestle. This mixture will give a Ph of 7.4.
5. Use doubly distilled water in the preparation of the above solutions.
6. The usual precautions should be taken in the cleaning of all glassware.

PROCEDURE:

1. Place 1000 c.c. of fresh doubly distilled water in pyrex 2 liter beaker.
2. Add 0.1 gm. of the prepared "buffer" mixture and thoroughly dissolve.
3. Now add 10 c.c. of 1 per cent potassium oxalate and mix thoroughly.
4. Place beaker on asbestos-filled wire gauze and heat until boiling over burner.
5. When the solution is boiling vigorously add 1.5 c.c. of 1 per cent potassium hydroxide.
6. Now while stirring rapidly, add 10 c.c. of 1 per cent gold chloride and continue boiling until reduction is complete. The resulting solution will be a beautiful cherry-red sol with extremely small particles.

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*Note:—The di-sodium hydrogen phosphate is usually furnished with 12 molecules of water of crystallization and must be dried to the two molecule form, $Na_2HPO_4+2H_2O$, before use. This is accomplished by drying in the paraffin oven at 56 degrees C. for 12 hours or by drying in the incubator at 37 degrees for two or three days. Care should be taken that the drying is not carried too far.

The following equations represent the chemical reactions involved in the preparation of colloidal gold by the above technic:

(1) $4\ AuCl_3 : HCl + 4\ KOH \rightarrow 4\ KCl + 4\ AuCl_3 + 4\ H_2O.$

(2) $4\ AuCl_3 + 6\ K_2C_2O_4 \rightarrow 4\ Au + 12\ KCl + 12\ CO_2.$

The dissociation of the "buffer" salts tends to equalize the charges on the gold particles and also maintains a constant Ph. In this respect its action is similar to that of a peptizing agent. The sensitivity of a gold sol depends upon the H-ion concentration, the salt concentration and the preferential adsorption of least hydrated ions. The "buffer" Ph should be approximately the same as that of the spinal fluid in order to prevent the accumulation of false charges on the protein particles.

Levinson[7] investigated the cause of the difference in H-ion concentration in fresh spinal fluids and those which had stood for a period of time. He observed the H-ion concentration in fresh fluids to range from 7.4 to 7.6; whereas after 24 hours fluids which stood uncorked showed Ph of 7.8 to 8.6. This difference he established as due to the escape of CO_2 into the air.

Mechanism of the Precipitation of Colloidal Gold by Spinal Fluid

From observations on the part of Weston,[8] it is readily conceded that the gold precipitating substance in the spinal fluid is contained in the globulin fraction and is of a protein nature. If such is the case, the author is led to believe that this substance behaves as an amphoteric electrolyte and exhibits typical colloidal properties because of the large size of the individual molecules. It must also possess an isoelectric point and according to the "Zwitter-ion" hypothesis of Bjerrum, carries an equal number of positive and negative charges due to complete dissociation of equal numbers of acid and basic groups in the molecule. In solutions acid to their isoelectric points, proteins exist as positively charged ions, capable of combining with negative ions to form salts, while in solutions alkaline to their isoelectric points, they exist as negatively charged ions which can combine only with positive ions. With respect to the Helmholtz double layer theory, it is logical to assume that the precipitation of colloidal gold by the cerebrospinal fluid is effected by mutual coagulation of colloids of opposite sign or adsorptive coagulation of colloids of like sign influenced by electrolytes.

Summary

A method for the preparation of colloidal gold is presented. The essential point in the technic is the control of the electrolytic forces involved, by the aid of a "buffer" mixture. A possible explanation of the mechanism of the precipitation of colloidal gold by cerebrospinal fluid is also given.

The author wishes to acknowledge the encouragement and advice received from Dr. Hamblen C. Eaton during the course of this work.

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A MODIFICATION OF VAN GIESEN'S CONNECTIVE-TISSUE STAIN

By PAUL A. MADER, B.S., M.T.

From the Clinical Laboratory of Drs. Brem, Zeiler, Hammack and Maier, Los Angeles, Calif.

The following technic has been devised for the purpose of establishing correct solutions and time elements for more consistent results in a connective-tissue stain. Particular attention has been paid to the picro-fuchsin so that sharp differentiation is attained without interference with the nuclear stain.

Weigert's iron hematoxylin should be used as the primary stain.

The picro-fuchsin is made as follows:

Saturated aqueous picric acid	30.0 c.c.
Aqueous acid fuchsin (1 per cent)	3.0 c.c.
Distilled water	67.0 c.c.

Either paraffin or frozen sections may be used. They should not be more than 10 microns thick.

1. Stain in hematoxylin for 5 minutes.

2. Wash in water for a few seconds. Where celloidin is employed, and if it is stained by the hematoxylin, a decolorization in 0.5 per cent. HcI in 70.0 per cent. alcohol is indicated. Otherwise this step may be eliminated.

3. Wash in running water for 5 minutes.

4. Counterstain in picro-fuchsin for 2 minutes.

5. Wash in 95 per cent. alcohol and blot.

6. Dehydrate in absolute alcohol.

7. Blot and clear with xylol.

8. Mount in balsam or gum damar.

PIONEERS IN MEDICAL TECHNOLOGY

Edward Jenner

(1749-1823)

Jenner, (pronounced Jen-ner), an English physician, was born at Berkeley, Gloucestershire. In his twenty-first year he went to London to study under the celebrated John Hunter.

He was the discoverer of one of the greatest triumphs in the history of medicine, the successful introduction of preventive inoculation against small-pox. It had long been a countryside tradition in England that dairy maids who had contracted cow-pox through milking did not take small-pox, and similar observations had been made in Germany and France. Jenner conceived the idea of applying this on a grand scale and in 1796 performed his first vaccination on a country boy, using material from the arm of a milk maid who had contracted cow-pox in the usual way. The crucial test was applied to the experiment by inoculating the boy with small-pox virus six weeks afterward and the immunity proved successful.

Although the evidence accumulated by Jenner seemed conclusive, the practice met with great opposition until a year had passed, when upwards of seventy of the principal physicians and surgeons in London signed a declaration of their confidence in it.

In 1798 he published his first memoir, entitled "An Inquiry into the Causes and Effects of the Variolae Vaccinae.

News and Announcements

BOARD OF REGISTRY

OF A. S. C. P.

During recent months, it has been called more and more to the attention of the Registry of Technicians that our certificate holders, either valuntarily or on request, are instructing students. Usually this takes the form of a "short course" of three or four months' duration. While the Laboratory Technician is sometimes forced into the role of teacher by pressure brought to bear by the hospital administration or for some protege of an influential staff member, as the Registry correspondence shows, on the other hand, we find that the technician herself, attracted by the additional remuneration, assumes this task which is the function of the approved training school. In either case, such instruction works to the detriment of the technician individually and to the group as a whole.

Many of our registrants have expressed their grievances and indignation when supplanted by unqualified workers, or upon realizing the incursion upon their own position when such students secure positions in their communities. The Registry appreciates the difficulties faced by the L.T., however, it is also true that laboratory directors and hospital administrators have been most willing to co-operate when the matter has been brought to their attention.

As the source is always the best point at which to attack an evil, we are appealing to the registered L.T.'s and M.T.'s to refuse such a task and help the Registry in eliminating teaching of this sort for the betterment of their own status, scientifically and economically, as well as for the good of the patients involved. The Board of Registry passed a ruling two years ago automatically invalidating the certificates of a registrant undertaking such teaching; and they are likewise willing to substantiate any registrant in his or her refusal to accept such an assignment. We are not unaware of the splendid co-operation of our membership and believe this to be an opportune time to bring the matter again to their attention and enlist their esprit de corps to work toward the goal of having qualified technicians taught only under proper auspices.

The final results of the April, 1935, examinations have been

tabulated. Four hundred and three applicants were awarded certificates having successfully passed their tests, and thirty-six did not make the passing grade.

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The 1935-36 Roster is now being prepared. All changes of address should be mailed into the office of the Registry immediately.

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An amendment has been proposed to the resolution passed by the Board of Registry at the June meeting, in Atlantic City, changing the qualifications for the rating of M.T. and increasing the number of eligibles. As soon as the referendum votes have been received and the final ruling enunciated a communication will be sent to all Laboratory Technicians apprising them of the procedure for having their certificates reclassified.

NATIONAL

VALLERY-RADOT, DR. PASTEUR, grandson of Pasteur, lectured at the Johns Hopkins Hospital on April 25 on "The Links between Pasteur's Discoveries" and on April 26 on "Experimental and Human Anaphylaxis."

MALLORY, DR. FRANK B., until his retirement in 1932 professor of pathology at the Harvard Medical School, editor of the American Journal of Pathology, was awarded the George M. Kober Medal by the Association of American Physicians at the recent Atlantic City meeting.

ZINSSER, DR. HANS, exchange professor from Harvard University to the University of Paris, is continuing his studies on typhus at the Pasteur Institute. He is working in a laboratory placed at his disposal by Professor Nicolle and is also giving lectures on bacteriology in the medical school.

FLEXNER, DR. SIMON, director of the laboratories of the Rockefeller Institute for Medical Research since its opening in 1903, has presented his resignation to take effect on the appointment of his successor.

Coming Events

The tenth International Congress of the History of Medicine will be held in Madrid from Sept. 23 to 29. Subjects for discussion will be: Arabian Medicine in Spain; Medicine in America during its Discovery and Colonization; and Medical Folk-lore in various Civilized Countries.

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May we call to your attention that the binder for Volumes 1 and

2 of the *Bulletin* can now be obtained through the administrative office. All orders received to date are being mailed.

STATE

Maryland

On May 25th the Maryland Society of Registered Technicians held a meeting at Mercy Hospital, Baltimore. As this was the last one of the fiscal year, the following officers were elected:

President—Edward P. Walker, M.T.

Vice-President—Mary E. Behr, L.T.

Secretary-Treasurer—Anne M. Gorman, L.T.

Board of Directors—Sr. M. Claude, L.T., Frances Donovan, L.T.

The Maryland Society is flourishing and the meetings are most interesting. At the May session J. Sheldon Eastland, M.D., presented a paper entitled, "Case Report of a Severe Diabetes in a Young Child" and Emil G. Schmidt, Ph.D., also of the University of Maryland presented a paper on the "Amino Acid Content of the Blood in Health and Disease." An interesting feature of every meeting is an open forum in which a member has the opportunity of presenting any special improvement in Laboratory Technique for the benefit of the other members or of asking for suggestions to smooth out some difficulty that he or she may be having.

Not only is there a good attendance at the meetings from the Maryland Society itself but several technicians from the D. of C. Society of Clinical Laboratory Technicians are always present and interested.

A September meeting is planned when delegates will report on the annual convention of the A. S. C. L. T. in Atlantic City.

Texas

Plans are under way for the Third Annual meeting of the Texas Society of Clinical Laboratory Technicians to be held in Houston, Texas, October 11th and 12th.

Scientific papers and exhibits will be presented by several members of the Society as well as various members of the medical profession.

Abstracts

DRIED BACTERIA, Science News, Science, Vol. 81, No. 2092, Feb. 1, 1935.

Dr. Alden F. Roe of the George Washington School of Medicine described how the technic of drying out certain bacterial types and still keeping them alive can be applied to a species that thrive only when they are not exposed to the air. The bacteria are grown in a suitable media, concentrated by centrifuging, the concentrated suspension of bacteria transferred to filter paper and dried rapidly in the cold under vacuum. The strips of filter paper are then transferred into small, sterile glass tubes, the air exhausted, and the tubes sealed until the bacteria are needed for experimental purposes.

HISTOLOGY, A SIMPLE DIFFERENTIAL STAIN FOR THE HUMAN HYPOPHYSIS, Charles Spark, M.D., Jour. Lab. & Clin. Med., Vol. 20, No. 5, P. 508-509, Feb. 1935.

The author claims to have obtained consistently satisfactory results with the method he describes. He gives the technic and the solutions used, the individual ingredients of which are available in any pathological laboratory. With this method he finds the various cellular elements of the human hypophysis sharply delineated and clearly defined from one another. Stained sections that are now two years old have not shown any significant degree of fading.

PAST DISCOVERIES

Manson discovered the transmission of filariasis by mosquitoes in 1879.

Pasteur discovered streptococcus, and pneumococcus in 1880.

Eberth isolated typhoid bacillus in 1880.

Book Reviews

BIOCHEMICAL LABORATORY METHODS FOR STUDENTS OF THE BIOLOGICAL SCIENCES. By Clarence Austin Morrow, Ph.D. Revised and rewritten by William Martin Sandstrom, Ph.D. Published by John Wiley & Sons, Inc., New York. 319 pages. Cloth binding, Price $3.75.

As the title suggests, the book was written primarily for the student as a laboratory handbook. Each item discussed is treated as a laboratory experiment and the principles involved are not taken into account. Many references are given following the experiments so that the student may gain the benefit of all the principles necessary and at the same time conserving space for more and varied experiments. Aside from being of value to the student, the organic and biological chemist will find much of value by having this volume in his collection of scientific works as a quick reference book for tests, preparations and identifications of many substances.

The following are among the many subjects: Colloidal solutions, emulsions, pH determinations, preparation of amino acids and derivatives, preparation of derived proteins, analysis of proteins, identification of mono- and disaccharides, preparation of sugars and derivatives, polysaccharide identification, preparation and quantitative determination of polysaccharides, detection of tannins and glucosides, acid- iodine- and thiocyanagen number of oils and fats, enzymes, and plant pigments.

CLINICAL LABORATORY MANUAL for Nurses and Technicians. By Sister Alma, chief laboratory technician and instructress of nurses at St. Thomas Hospital, Akron, Ohio. C. V. Mosby, St. Louis, Publishers, 1932. Cloth, illustrated, pp. 159. Price $1.75.

All of us who are responsible for instructing nurses or securing the co-operation of the hospital staff with the laboratory, will find this little book indispensable.

The first part deals in explicit and simple fashion with the problem of getting specimens to the laboratory in proper condition for examination, and of reporting results. This subject is dealt with very completely and Sister Alma's ready wit brightens a text which from a heavier pen would surely have proved dull.

The second part of the book deals with laboratory methods and covers quite adequately the methods in use in the usual clinical laboratory. A list of reagents and solutions follows.

Not only technicians and nurses, but also the physician and the interne will find many valuable hints in this volume for improving the laboratory service.

ILLUSTRATIONS OF REGIONAL ANATOMY, by E. B. Jamieson, M.D., Senior Demonstrator and Lecturer, Anatomy Department, University of Edinburgh. William Wood and Co., Baltimore, Publishers. 5 volumes, 1934. Price $9.00.

The technician who attends post mortems and who wishes to know some anatomy for the good of her soul, can find no more delightful and practical way of learning it than by poring over these plates.

The five little volumes are bound in loose-leaf folders so that supplementary sheets may be added later. They cover central nervous system, head and neck, abdomen, pelvis, and thorax. They are a series of semidiagramatic drawings, most of them in color, which Dr. Jamieson has used in teaching his classes in Edinburgh. Those of us who are fortunate enough to have studied under them are glad that he is taking this opportunity of broadening his public and "telling the world" in this graphic fashion about the structure of the human body.

The names used in the legends are those adopted by the Anatomical Society of Great Britain but wherever they differ radically from the B.N.A. terminology, the latter names are included in brackets. No text accompanies the illustrations.